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L68  ANSWER 1 OF 18  USPATFULL on STN
ACCESSION NUMBER:         2003:250499  USPATFULL
TITLE:                    Molecule of pharmaceutical interest comprising at its
                          n-terminal a glutamic acid or a glutamine in the form
                          of a physiologically acceptable strong acid
```

INVENTOR(S): Klinguer-Hamour, Christine, Groisy, FRANCE
Nathalie, Corvaia, Genevois, FRANCE
Alain, Beck, Saleve, FRANCE
Liliane, Goetsch, Ayze, FRANCE

	NUMBER	KIND	DATE	
PATENT INFORMATION:	US 2003175285	A1	20030918	
APPLICATION INFO.:	US 2002-239313	A1	20020919	(10)
	WO 2001-FR872		20010322	

	NUMBER	DATE
PRIORITY INFORMATION:	FR 2000-3711	20000323

DOCUMENT TYPE: Utility
FILE SEGMENT: APPLICATION
LEGAL REPRESENTATIVE: THE FIRM OF HUESCHEN AND SAGE, 500 COLUMBIA PLAZA, 350 EAST MICHIGAN AVENUE, KALAMAZOO, MI, 49007
NUMBER OF CLAIMS: 30
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 4 Drawing Page(s)
LINE COUNT: 3318
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The invention concerns a molecule of pharmaceutical interest, preferably a major histocompatibility complex (MHC) ligand, comprising a glutamic acid or a glutamine at its N-terminal, in the form of a physiologically acceptable addition salt, and a vaccine comprising such a ligand.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L68 ANSWER 2 OF 18 IFIPAT COPYRIGHT 2004 IFI on STN
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TITLE: FUNCTIONAL ROLE OF ADRENOMEDULLIN (AM) AND THE GENE-RELATED PRODUCT (PAMP) IN HUMAN PATHOLOGY AND PHYSIOLOGY; PEPTIDE FOR USE IN THE DIAGNOSIS, TREATMENT AND PREVENTION OF INFECTIONS, CANCER, DIABETES AND SKIN DISORDERS; WOUNG HEALING AGENTS, ANTICARCINOGENEIC AGENTS
INVENTOR(S): Cuttitta; Frank, Adamstown, MD, US
Gray; Karen, Gaithersburg, MD, US
Hook; William, Wheaton, MD, US
Macri; Charles, Kensington, MD, US
Martinez; Alfredo, McLean, VA, US
Miller; Mae Jean, Monrovia, MD, US
Unsworth; Edward J., Kensington, MD, US
Walsh; Thomas, Bethesda, MD, US
PATENT ASSIGNEE(S): Unassigned
AGENT: MORGAN & FINNEGAN, L.L.P., 345 Park Avenue, New York, NY, 10154-0053, US

	NUMBER	PK	DATE
PATENT INFORMATION:	US 2002055615	A1	20020509
APPLICATION INFORMATION:	US 2001-931700		20010816

	APPLN. NUMBER	DATE	GRANTED PATENT NO. OR STATUS
DIVISION OF:	US 1998-11922	19980217	6320022

	NUMBER	DATE	
PRIORITY APPLN. INFO.:	WO 1996-US13286	19960816	
	US 1995-2514P	19950818	(Provisional)
	US 1995-2936P	19950830	(Provisional)
	US 1996-13172P	19960312	(Provisional)

FAMILY INFORMATION:	US 2002055615 20020509
	US 6320022
DOCUMENT TYPE:	Utility
	Patent Application - First Publication
FILE SEGMENT:	CHEMICAL
	APPLICATION
NUMBER OF CLAIMS:	16 27 Figure(s).

DESCRIPTION OF FIGURES:

FIG. 1: FIG. 1 sets forth a schematic drawing showing the structures of the human AM gene, mRNA, and preprohormone containing the two biologically active molecules, AM and pro-AM peptide (PAMP). The positions of the oligonucleotides and peptides synthesized are shown. Numbers in the gene and mRNA indicate base pairs from the initiation codon. Numbers in the protein correspond to amino acids. Data are modified from the report of Ishimitsu, et al., Biochem Biophys Res Commun 203:631639 (1994).

FIG. 2: FIG. 2 sets forth a titration curve for rabbit anti-PO72 immunogen (bleed 2343) binding to solid phase test peptides. A measurable antibody interaction was observed in AM, PO72, PO71, NPY, and CGRP. All other target peptides (PO70, gastrinreleasing peptide, glucagon-like peptide 1, vasoactive intestinal peptide, arginine vasopressin, GRF, cholecystokinin, gastrin, oxytocin, calcitonin, alpha MSH, and BSA) showed negligible binding.

FIG. 3: Detection of AM-like immunoreactive species in the whole cell lysate of a human lung carcinoid cell line, NCI-H720. The right lane contains 2 ng synthetic PO72 (molecular weight, 3576) . The specificity of detection is demonstrated by antigen absorption of anti-PO72 antiserum (right panel).

FIGS. 4A, 4B, 4C, and 4D: FIGS. 4A-4D set forth a cross-section (magnification x 450) of a bronchiolus showing immunoreactivity to the anti-AM antiserum in the epithelium (FIG. 4A) and labeling of the AM mRNA after in situ RT-PCR (FIG. 4C). Absorption controls (FIG. 4B) and omission of the RT (FIG. 4D) confirmed the specificity of the staining.

FIGS. 5A, 5B, 5C, and 5D: FIGS. 5A-5D set forth photographs of a section through the adventitia layer of a bronchus showing a small nervous ganglion where the perykaria of the neurons and some nerves are immunostained (FIG. 5A), whereas a serial section treated with preabsorbed antiserum was negative (FIG. 5B). (Magnification x 450). Another ganglion appears labeled, at lower magnification (x 120), after application of the in situ RT-PCR technique (FIG. 5C). Arrows point to blood vessels whose endothelial layers are clearly positive. Omission of primers in the PCR mixture gave negative staining (FIG. 5D).

FIGS. 6A and 6B: FIG. 6A and 6B set forth photographs of the detail of ***chondrocytes*** immunostained with anti-AM (FIG. 6A) and with the antiserum preabsorbed with PO72 (FIG. 6B). (Magnification x 700).

FIG. 7A and 7B: FIGS. 7A and 7B set forth photographs of alveolar macrophages labeled for AM mRNA after in situ RT-PCR (FIG. 7A) and negative control without reverse transcriptase (FIG. 7B). (Magnification x 450).

FIG. 8: Characterization of AM by RT-PCR in mRNA from normal tissues and pulmonary tumor cell lines. The PCR products had the proper size (410 bp) when visualized with UV light (lower panel), and they hybridized with the specific probe after Southern blot (upper panel). H146 and H345 are small cell carcinomas, H676 is an adenocarcinoma, H720 is a carcinoid, and H820 is a bronchioalveolar carcinoma. H146 was the only cell line that tested negative for AM.

FIGS. 9A and 9B: FIGS. 9A and 9B set forth photographs of cell line H820 (bronchioalveolar carcinoma) showing a cytoplasmic distribution of AM mRNA, as revealed by in situ RT-PCR (FIG. 9A) , and a serial section demonstrating that the staining disappears when the reverse transcription step is omitted (FIG. 9B). (Magnification x 550)

FIGS. 10A and 10B: FIGS. 10A and 10B set forth photographs of serial sections of an adenocarcinoma showing AM mRNA in the tumor cells by in situ RT-PCR (FIG. 10A) and immunocytochemistry (FIG. 10B). (Magnification x 550)

FIG. 11: FIG. 11 sets forth a chart indicating histamine release from rat mast cells.

FIGS. 12A and 12B: FIGS. 12A and 12B indicate the effect of antiAM MoAb on the growth of human tumor cell lines.

FIG. 13: FIG. 13 sets forth a characterization of the monoclonal antibody MoAb-G6 showing binding to AM (composite-function) and to two PO72 molecules:

an in-house peptide (circle-solid) and a Peninsula peptide product (*). All other peptides: PO70, GRP, GLP1, VIP, AVP, GRF, CCK, **amylin**, gastrin, oxytocin, AMSH, pancreatic polypeptide, peptide YY, Taa-HoTH (Tabanus atratus Hypotrehalosemic Hormone), and BSA, showed negligible binding. Solid-phase assays were conducted using previously described methods (Cuttitta, et al., Nature 316, 823 (1985)).

FIGS. 14A, 14B, 14C and 14D: FIGS. 14A and 14B show a representative sample of human tumor cell lines (H157, H720, MCF-7, OVCAR-3, SNUC-1) and normal human tissues (brain, lung, heart, adrenal) screened for AM mRNA and its translated protein. FIG. 14A is a Southern blot analysis and FIG. 14B is the ethidium bromide 1% agarose gel which demonstrates the predicted 410 bp product for AM mRNA as evaluated by RT-PCR analysis. FIG. 14C sets forth a Western blot analysis showing immunoreactive species of 18, 14, and 6 kDa when using a rabbit antiserum to AM.

FIGS. 15A, 15B and 15C: FIGS. 15A-15C set forth an HPLC profile, solid phase plate assay and Western blot analysis of H720 conditioned medium (CM). FIG. 15A illustrates the fractionation of 5 L of H720 CM compared with the elution time of synthetic AM at 89.4 min (arrow).

FIGS. 16A, 16B, 16C and 16D: A representative human tumor cell line, MCF-7, was used to show the growth effects, cAMP activity and receptor binding by AM under serumfree, hormone-free conditions. FIG. 16A shows the inhibitory effects of MoAb-G6 (circle-solid) compared with no effect from its mouse myeloma isotypic control, IgAK (Sigma) (composite-function). FIG. 16B shows that the effects of MoAb-G6 were overcome by the addition of synthetic AM (composite-function) compared with the addition of AM alone (circle-solid). FIG. 16C indicates that cyclic AMP is activated with the addition of synthetic AM. FIG. 16D shows that specific receptor binding is higher for AM (composite-function) than for PAMP (*) or PO72 (circle-solid). MTT (Carney, et al., Proc. Natl. Acad Sci. U.S.A. 79, 3185 (1981)) and receptor binding/cAMP assay techniques (T. W. Moody, et al., Proc. Natl. Acad. Sci U.S.A. 90, 4345 (1993)) are described elsewhere.

FIGS. 17A-17H: FIGS. 17A-17H set forth the distribution of adrenomedullin (AM) in the pancreas as shown by immunocytochemistry.

FIGS. 18A and 18B: Effects of AM and MoAb-G6 (alpha-AM) on the release of insulin from rat isolated islets. (FIG. 18A) Increasing concentration of AM reduces insulin secretion in the presence (composite-function) or absence (circle-solid) of MoAb-G6 antibody. Note dramatic increase in insulin secretion mediated by the antibody. (FIG. 18B)

FIGS. 19A and 19B: FIG. 19A shows a Southern blot for AM in six cell lines expressing insulin and in human adrenal and pancreas mRNA. FIG. 19B shows the same gel as seen by UV before transfer.

FIGS. 20A and 20B: Glucose tolerance tests were performed on Sprague-Dawley rats (250 to 300 g) in the presence (compositefunction) or absence (circle-solid) of AM.

FIGS. 21A-21I: FIG. 21 sets forth in panels A-I the localization of AM mRNA and immunoreactivity in various organs of different species. Panel A shows mRNA for AM detected by in situ RT-PCR in the epithelial cells of the rat trachea. Panel B sets forth guinea pig trachea displaying a strong immunoreactivity to the AM antibody, specially in the apical region. Panel C depicts a Xenopus respiratory tract, with intense immunostaining in the supranuclear region. Panel D shows Xenopus integument with AM immunoreactivity concentrated in the unicellular glands of the epidermis (two of which appear in this figure). The dark spot to the left is a chromatophore. Panel E shows skin of a 16-day old mouse embryo. An intense immunoreactivity to AM is observed in the epidermis and in the subjacent developing muscles. Panel F sets forth a hamster uterus showing immunostaining for AM in both the lining epithelium and the glands. Panel G shows a small salivary gland found in the hamster tongue. Discrete secretory cells store the AM-like material. Panel R shows rat duodenum with intensely immunostained Brunner's glands. Panel I shows a section of cat colon containing an AM-positive endocrine cell.

FIG. 22; FIG. 22 indicates the effect of AM and PAMP on the inhibition of growth of E. coli. AM demonstrated higher growth inhibitory activity than albumin (Alb) (negative control) (*, p=0.03), PO70 (pilcrow, p=0.04), PO71 (pilcrow, p=0.006), and PO72 (pilcrow, p=0.03). Magainin (M) exerted greater inhibitory activity against E. coli than did AM (* pilcrow section dagger-relation, p=0.03) and PAMP (section daggerrelation, p=0.009). Data were compiled from 14 experiments.

FIGS. 23A and 23B: FIGS. 23A and 23B set forth the antimicrobial activity of AM and PAMP.
FIG. 24: FIG. 24 indicates the effect of AM on the germination of C. albicans.
FIG. 25: FIG. 25 sets forth the distribution of amphipathic regions for AM and PAMP as calculated for a-helix/b-sheet angle parameters (Eisenberg), and the helical wheel projection display for AM and PAMP (DNASTAR).
FIGS. 26A-26D: FIG. 26 sets forth a representative sample of human tumor cell lines and normal human tissues screened for AM and AM-R. Southern blot analysis demonstrates the predicted 410 bp product for AM (A) and a 471 bp product for AM-R mRNA (B) after RT-PCR amplification. (C) Western blot analysis of cell extracts shows immunoreactive species of 18, 14, and 6 kDa when using a rabbit antiserum to AM. In addition, there is a 22 kDa immunoreactive entity that may be attributed to posttranslational processing. (D) The absorption control was negative.

DESCRIPTION OF FIGURES:

FIGS. 27A-27D: FIG. 27 sets forth the iumunohistochemical and in situ RT-PCR analysis of human cancer cell lines for AM. (A) Immunohistochemical analysis for AM in SCLC H774 and (B) ovarian carcinoma cell line NIH: Ovcar-3. Note the peripheral distribution of AM immunoreactivity in H774 colonies. (C) In situ RT-PCR for AM mRNA in carcinoid cell line H720 and (D) negative control in a serial section where primers were substituted by water in the PCR mixture. !

AB The methods of the present invention demonstrate that adrenomedullin (AM) is expressed in human cancer cell lines of diverse origin and functions as a universal autocrine growth factor driving neoplastic proliferation. The present invention provides for Tpeptides and AM antibodies useful in therapeutic, pharmacologic and physiologic compositions. The present invention additionally provides for methods of diagnosis, treatment and prevention of disease utilizing compositions comprising the AM peptides and antibodies of the present invention. The methods of the present invention also provide for experimental models for use in identifying the role of AM in pancreatic physiology. The methods pertaining to rat isolated islets have shown that AM inhibits insulin secretion in a dose-dependent manner. The monoclonal antibody MoAb-G6, which neutralizes AM bioactivity, was shown by the methods of the present invention to increase insulin release fivefold, an effect that was reversed by the addition of synthetic AM.

CLMN 16 27 Figure(s).

FIG. 1: FIG. 1 sets forth a schematic drawing showing the structures of the human AM gene, mRNA, and preprohormone containing the two biologically active molecules, AM and pro-AM peptide (PAMP). The positions of the oligonucleotides and peptides synthesized are shown. Numbers in the gene and mRNA indicate base pairs from the initiation codon. Numbers in the protein correspond to amino acids. Data are modified from the report of Ishimitsu, et al., Biochem Biophys Res Commun 203:631639 (1994).

FIG. 2: FIG. 2 sets forth a titration curve for rabbit anti-PO72 immunogen (bleed 2343) binding to solid phase test peptides. A measurable antibody interaction was observed in AM, PO72, PO71, NPY, and CGRP. All other target peptides (PO70, gastrinreleasing peptide, glucagon-like peptide 1, vasoactive intestinal peptide, arginine vasopressin, GRF, cholecystokinin, gastrin, oxytocin, calcitonin, alpha MSH, and BSA) showed negligible binding.

FIG. 3: Detection of AM-like immunoreactive species in the whole cell lysate of a human lung carcinoid cell line, NCI-H720. The right lane contains 2 ng synthetic PO72 (molecular weight, 3576) . The specificity of detection is demonstrated by antigen absorption of anti-PO72 antiserum (right panel).

FIGS. 4A, 4B, 4C, and 4D: FIGS. 4A-4D set forth a cross-section (magnification x 450) of a bronchiolus showing immunoreactivity to the anti-AM antiserum in the epithelium (FIG. 4A) and labeling of the AM mRNA after in situ RT-PCR (FIG. 4C). Absorption controls (FIG. 4B) and omission of the RT (FIG. 4D) confirmed the specificity of the staining.

FIGS. 5A, 5B, 5C, and 5D: FIGS. 5A-5D set forth photographs of a section through the adventitia layer of a bronchus showing a small nervous ganglion where the perykaria of the neurons and some nerves are immunostained (FIG. 5A), whereas a serial section treated with

preabsorbed antiserum was negative (FIG. 5B). (Magnification x 450). Another ganglion appears labeled, at lower magnification (x 120), after application of the in situ RT-PCR technique (FIG. 5C). Arrows point to blood vessels whose endothelial layers are clearly positive. Omission of primers in the PCR mixture gave negative staining (FIG. 5D).

FIGS. 6A and 6B: FIG. 6A and 6B set forth photographs of the detail of **chondrocytes** immunostained with anti-AM (FIG. 6A) and with the antiserum preabsorbed with PO72 (FIG. 6B). (Magnification x 700).

FIG. 7A and 7B: FIGS. 7A and 7B set forth photographs of alveolar macrophages labeled for AM mRNA after in situ RT-PCR (FIG. 7A) and negative control without reverse transcriptase (FIG. 7B). (Magnification x 450).

FIG. 8: Characterization of AM by RT-PCR in mRNA from normal tissues and pulmonary tumor cell lines. The PCR products had the proper size (410 bp) when visualized with UV light (lower panel), and they hybridized with the specific probe after Southern blot (upper panel). H146 and H345 are small cell carcinomas, H676 is an adenocarcinoma, H720 is a carcinoid, and H820 is a bronchioalveolar carcinoma. H146 was the only cell line that tested negative for AM.

FIGS. 9A and 9B: FIGS. 9A and 9B set forth photographs of cell line H820 (bronchioalveolar carcinoma) showing a cytoplasmic distribution of AM mRNA, as revealed by in situ RT-PCR (FIG. 9A) , and a serial section demonstrating that the staining disappears when the reverse transcription step is omitted (FIG. 9B). (Magnification x 550)

FIGS. 10A and 10B: FIGS. 10A and 10B set forth photographs of serial sections of an adenocarcinoma showing AM mRNA in the tumor cells by in situ RT-PCR (FIG. 10A) and immunocytochemistry (FIG. 10B). (Magnification x 550)

FIG. 11: FIG. 11 sets forth a chart indicating histamine release from rat mast cells.

FIGS. 12A and 12B: FIGS. 12A and 12B indicate the effect of antiAM MoAb on the growth of human tumor cell lines.

FIG. 13: FIG. 13 sets forth a characterization of the monoclonal antibody MoAb-G6 showing binding to AM (composite-function) and to two PO72 molecules: an in-house peptide (circle-solid) and a Peninsula peptide product (*). All other peptides: PO70, GRP, GLP1, VIP, AVP, GRF, CCK, **amylin**, gastrin, oxytocin, AMSH, pancreatic polypeptide, peptide YY, Taa-HoTH (Tabanus atratus Hypotrehalosemic Hormone), and BSA, showed negligible binding. Solid-phase assays were conducted using previously described methods (Cuttitta, et al., Nature 316, 823 (1985)).

FIGS. 14A, 14B, 14C and 14D: FIGS. 14A and 14B show a representative sample of human tumor cell lines (H157, H720, MCF-7, OVCAR-3, SNUC-1) and normal human tissues (brain, lung, heart, adrenal) screened for AM mRNA and its translated protein. FIG. 14A is a Southern blot analysis and FIG. 14B is the ethidium bromide 1% agarose gel which demonstrates the predicted 410 bp product for AM mRNA as evaluated by RT-PCR analysis. FIG. 14C sets forth a Western blot analysis showing immunoreactive species of 18, 14, and 6 kDa when using a rabbit antiserum to AM.

FIGS. 15A, 15B and 15C: FIGS. 15A-15C set forth an HPLC profile, solid phase plate assay and Western blot analysis of H720 conditioned medium (CM). FIG. 15A illustrates the fractionation of 5 L of H720 CM compared with the elution time of synthetic AM at 89.4 min (arrow).

FIGS. 16A, 16B, 16C and 16D: A representative human tumor cell line, MCF-7, was used to show the growth effects, cAMP activity and receptor binding by AM under serumfree, hormone-free conditions. FIG. 16A shows the inhibitory effects of MoAb-G6 (circle-solid) compared with no effect from its mouse myeloma isotypic control, IgAK (Sigma) (composite-function). FIG. 16B shows that the effects of MoAb-G6 were overcome by the addition of synthetic AM (composite-function) compared with the addition of AM alone (circle-solid). FIG. 16C indicates that cyclic AMP is activated with the addition of synthetic AM. FIG. 16D shows that specific receptor binding is higher for AM (composite-function) than for PAMP (*) or PO72 (circle-solid). MTT (Carney, et al., Proc. Natl. Acad Sci. U.S.A. 79, 3185 (1981)) and receptor binding/cAMP assay techniques (T. W. Moody, et al., Proc. Natl. Acad. Sci U.S.A. 90, 4345 (1993)) are described elsewhere.

FIGS. 17A-17H: FIGS. 17A-17H set forth the distribution of adrenomedullin (AM) in the pancreas as shown by immunocytochemistry.

FIGS. 18A and 18B: Effects of AM and MoAb-G6 (alpha-AM) on the release of insulin from rat isolated islets. (FIG. 18A) Increasing concentration of AM reduces insulin secretion in the presence (composite-function) or absence (circle-solid) of MoAb-G6 antibody. Note dramatic increase in insulin secretion mediated by the antibody. (FIG. 18B)

FIGS. 19A and 19B: FIG. 19A shows a Southern blot for AM in six cell lines expressing insulin and in human adrenal and pancreas mRNA. FIG. 19B shows the same gel as seen by UV before transfer.

FIGS. 20A and 20B: Glucose tolerance tests were performed on Sprague-Dawley rats (250 to 300 g) in the presence (compositefunction) or absence (circle-solid) of AM.

FIGS. 21A-21I: FIG. 21 sets forth in panels A-I the localization of AM mRNA and immunoreactivity in various organs of different species. Panel A shows mRNA for AM detected by in situ RT-PCR in the epithelial cells of the rat trachea. Panel B sets forth guinea pig trachea displaying a strong immunoreactivity to the AM antibody, specially in the apical region. Panel C depicts a Xenopus respiratory tract, with intense immunostaining in the supranuclear region. Panel D shows Xenopus integument with AM immunoreactivity concentrated in the unicellular glands of the epidermis (two of which appear in this figure). The dark spot to the left is a chromatophore. Panel E shows skin of a 16-day old mouse embryo. An intense immunoreactivity to AM is observed in the epidermis and in the subjacent developing muscles. Panel F sets forth a hamster uterus showing immunostaining for AM in both the lining epithelium and the glands. Panel G shows a small salivary gland found in the hamster tongue. Discrete secretory cells store the AM-like material. Panel R shows rat duodenum with intensely immunostained Brunner's glands. Panel I shows a section of cat colon containing an AM-positive endocrine cell.

FIG. 22; FIG. 22 indicates the effect of AM and PAMP on the inhibition of growth of E. coli. AM demonstrated higher growth inhibitory activity than albumin (Alb) (negative control) (*, p=0.03), PO70 (pilcrow, p=0.04), PO71 (pilcrow, p=0.006), and PO72 (pilcrow, p=0.03). Magainin (M) exerted greater inhibitory activity against E. coli than did AM (* pilcrow section dagger-relation, p=0.03) and PAMP (section daggerrelation, p=0.009). Data were compiled from 14 experiments.

FIGS. 23A and 23B: FIGS. 23A and 23B set forth the antimicrobial activity of AM and PAMP.

FIG. 24: FIG. 24 indicates the effect of AM on the germination of C. albicans.

FIG. 25: FIG. 25 sets forth the distribution of amphipathic regions for AM and PAMP as calculated for a-helix/b-sheet angle parameters (Eisenberg), and the helical wheel projection display for AM and PAMP (DNASTAR).

FIGS. 26A-26D: FIG. 26 sets forth a representative sample of human tumor cell lines and normal human tissues screened for AM and AM-R. Southern blot analysis demonstrates the predicted 410 bp product for AM (A) and a 471 bp product for AM-R mRNA (B) after RT-PCR amplification. (C) Western blot analysis of cell extracts shows immunoreactive species of 18, 14, and 6 kDa when using a rabbit antiserum to AM. In addition, there is a 22 kDa immunoreactive entity that may be attributed to posttranslational processing. (D) The absorption control was negative.

FIGS. 27A-27D: FIG. 27 sets forth the iumunohistochemical and in situ RT-PCR analysis of human cancer cell lines for AM. (A) Immunohistochemical analysis for AM in SCLC H774 and (B) ovarian carcinoma cell line NIH: Ovcar-3. Note the peripheral distribution of AM immunoreactivity in H774 colonies. (C) In situ RT-PCR for AM mRNA in carcinoid cell line H720 and (D) negative control in a serial section where primers were substituted by water in the PCR mixture. !

L68 ANSWER 3 OF 18 USPATFULL on STN

ACCESSION NUMBER: 2002:152617 USPATFULL

TITLE: Glucose-dependent insulinotropic peptide for use as an osteotropic hormone

INVENTOR(S): Isales, Carlos M., 3413 Woodstone Pl., Augusta, GA,

United States 30909
Bollag, Roni J., 231 Watervale Rd., Martinez, GA, United States 30907
Rasmussen, Howard, 820 Barrett La., Augusta, GA, United States 30909

	NUMBER	KIND	DATE
PATENT INFORMATION:	US 6410508	B1	20020625
APPLICATION INFO.:	US 1999-414189		19991007 (9)

	NUMBER	DATE
PRIORITY INFORMATION:	US 1998-103495P	19981008 (60)
	US 1998-103333P	19981007 (60)

DOCUMENT TYPE: Utility
FILE SEGMENT: GRANTED
PRIMARY EXAMINER: Priebe, Scott D.
ASSISTANT EXAMINER: Kaushal, Sumesh
LEGAL REPRESENTATIVE: Rothschild, Esq, Cynthia B., Kilpatrick Stockton LLP
NUMBER OF CLAIMS: 13
EXEMPLARY CLAIM: 1
NUMBER OF DRAWINGS: 15 Drawing Figure(s); 13 Drawing Page(s)
LINE COUNT: 1515
CAS INDEXING IS AVAILABLE FOR THIS PATENT.

AB The examples demonstrate that GIP receptor mRNA and protein are present in normal bone and osteoblastic-like cell lines, and that high-affinity receptors for GIP can be demonstrated by .sup.125I GIP binding studies. When applied to osteoblast-like cells (SaOS2), GIP stimulated an increase in cellular cAMP content and in intracellular calcium, with both responses being dose dependent. Moreover, administration of GIP results in elevated expression of collagen type I mRNA as well as an increase in alkaline phosphatase activity. Both of these effects reflect anabolic actions of presumptive osteoblasts. These results provide the first evidence that GIP receptors are present in bone and osteoblastic like cells and that GIP modulates the function of these cells. GIP has anabolic actions on remodeling bone, increasing vertebral bone density in a rat model of osteoporosis. GIP at 10 nM inhibits PTH-induced bone resorption in a fetal long bone assay and stimulates the synthesis of type 1 collagen mRNA. Transgenic mice overexpressing GIP have increased bone density compared to same age controls. GIP or analoges thereof can therefore be used as a therapeutic to inhibit bone resorption and to maintain or increase bone density. GIP antagonists, compounds which block binding to the GIP receptor, can be used to decrease bone density.

CAS INDEXING IS AVAILABLE FOR THIS PATENT.

L68 ANSWER 4 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 1
ACCESSION NUMBER: 2002:697027 CAPLUS
DOCUMENT NUMBER: 138:377
TITLE: Amylin and adrenomedullin: novel regulators of bone growth
AUTHOR(S): Cornish, J.; Naot, D.
CORPORATE SOURCE: Department of Medicine, University of Auckland, Auckland, N. Z.
SOURCE: Current Pharmaceutical Design (2002), 8(23), 2009-2021
CODEN: CPDEFP; ISSN: 1381-6128
PUBLISHER: Bentham Science Publishers
DOCUMENT TYPE: Journal; General Review
LANGUAGE: English

AB A review. Bone growth is regulated by circulating hormones and locally generated factors. Understanding their mechanisms of action enables us to obtain a better appreciation of the cellular and mol. basis of bone remodelling, and could therefore be valuable in approaches to new therapies. In this review, we consider the actions on bone tissue of the peptide hormones amylin and adrenomedullin, known to circulate at

picomolar concns. Adrenomedullin is also produced locally in bone. Amylin and adrenomedullin are related peptides with some homol. to both calcitonin and calcitonin gene-related peptide. These peptides have recently been found to stimulate the proliferation of osteoblasts in vitro, and to increase indexes of bone formation when administered either locally or systemically in vivo. In addition, amylin inhibits bone resorption. Both **amylin** and adrenomedullin have also been found to act on **chondrocytes**, stimulating their proliferation in culture and increasing tibial growth plate thickness when administered systemically to adult mice. Like the peptides themselves, the receptors for the calcitonin family are also related to each other. Each peptide seems to act through its own distinct high affinity receptor, as well as through other receptors for the family, usually with lower affinity. Characterization of the putative receptors expressed in osteoblasts, has provided some understanding of the physiol. effects of amylin and adrenomedullin in these cells. Studies of structure-activity relationships have demonstrated that osteotropic effects of amylin and adrenomedullin can be retained in peptide fragments of the mol. while losing the parent mol.'s effects on carbohydrate metabolism or vasodilatory properties resp. Thus, these small peptides, or their analogs, are attractive candidates as anabolic therapies for osteoporosis.

REFERENCE COUNT: 76 THERE ARE 76 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L68 ANSWER 5 OF 18 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 2002:252814 SCISEARCH
THE GENUINE ARTICLE: 530FB
TITLE: The **chondrocyte** is a target cell for **amylin** and adrenomedullin
AUTHOR: Naot D (Reprint); Cornish J; Callon K E; Poole C A; Lin C Q X; Xiao C L; Bava U; Clatworthy M; Cooper G J S; Reid I R
CORPORATE SOURCE: Univ Auckland, Dept Med, Auckland, New Zealand
COUNTRY OF AUTHOR: New Zealand
SOURCE: BONE, (MAR 2002) Vol. 30, No. 3, Supp. [S], pp. 30S-30S. MA C118.
Publisher: ELSEVIER SCIENCE INC, 655 AVENUE OF THE AMERICAS, NEW YORK, NY 10010 USA.
ISSN: 8756-3282.
DOCUMENT TYPE: Conference; Journal
LANGUAGE: English
REFERENCE COUNT: 0

L68 ANSWER 6 OF 18 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
DUPLICATE 2
ACCESSION NUMBER: 2003:58689 BIOSIS
DOCUMENT NUMBER: PREV200300058689
TITLE: Effects of amylin and adrenomedullin on the skeleton.
AUTHOR(S): Cornish, J. [Reprint Author]; Reid, I. R.
CORPORATE SOURCE: Department of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand
j.cornish@auckland.ac.nz
SOURCE: Journal of Musculoskeletal & Neuronal Interactions, (September 2001) Vol. 2, No. 1, pp. 15-24. print.
ISSN: 1108-7161 (ISSN print).
DOCUMENT TYPE: Article
General Review; (Literature Review)
LANGUAGE: English
ENTRY DATE: Entered STN: 22 Jan 2003
Last Updated on STN: 22 Jan 2003

AB Amylin and adrenomedullin are related peptides with some homology to both calcitonin and calcitonin gene-related peptide (CGRP). All these peptides have in common a 6-amino acid ring structure at the amino-terminus created by a disulfide bond. In addition, the carboxy-termini are amidated. Both amylin and adrenomedullin have recently been found to stimulate the proliferation of osteoblasts in vitro, and to increase indices of bone

formation in vivo when administered either locally or systemically. Both **amylin** and adrenomedullin have also been found to act on **chondrocytes** (Cornish et al., submitted for publication), stimulating their proliferation in culture and increasing tibial growth plate thickness when administered systemically to adult mice. Studies of structure-activity relationships have demonstrated that osteotropic effects of amylin and adrenomedullin can be retained in peptide fragments of the molecules. The full-length peptide of amylin has known effects on fuel metabolism, and systemic administration of amylin is also associated with increased fat mass. However, the octapeptide fragment of the molecule, amylin-(1-8), is osteotropic and yet has no activity on fuel metabolism. Similar fragments of adrenomedullin have also been defined, which retain activity on bone but lack the parent peptide's vasodilator properties. Both amylin-(1-8) and adrenomedullin-(27-52) act as anabolic agents on bone, increasing bone strength when administered systemically. Thus, these small peptides, or analogues of it, are potential candidates as anabolic therapies for osteoporosis. Both amylin and adrenomedullin may have effects on bone metabolism. Amylin is secreted following eating and may direct calcium and protein absorbed from the meal into new bone synthesis. Amylin circulates in high concentrations in obese individuals, and might contribute to the association between bone mass and fat mass. Our recent findings demonstrating the co-expression of adrenomedullin and adrenomedullin receptors in ostcoblasts, along with the findings that the peptide and its receptor are easily detectable during rodent embryogenesis, suggest that this peptide is a local regulator of bone growth. Thus, the findings reviewed in this 'paper illustrate that amylin and adrenomedullin may be relevant to the normal regulation of bone mass and to the design of agents for the treatment of osteoporosis.

L68 ANSWER 7 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 3
ACCESSION NUMBER: 1999:233770 CAPLUS
DOCUMENT NUMBER: 130:247465
TITLE: Stimulation of **chondrocyte** proliferation by **amylin** and adrenomedullin
INVENTOR(S): Reid, Ian Reginald; Cornish, Jillian
PATENT ASSIGNEE(S): Auckland Uniservices Limited, N. Z.
SOURCE: PCT Int. Appl., 25 pp.
CODEN: PIXXD2
DOCUMENT TYPE: Patent
LANGUAGE: English
FAMILY ACC. NUM. COUNT: 1
PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 9916406	A2	19990408	WO 1998-NZ145	19980925
WO 9916406	A3	19990708		
W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG				
EP 1027027	A2	20000816	EP 1998-946738	19980925
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				
JP 2001524454	T2	20011204	JP 2000-513546	19980925

PRIORITY APPLN. INFO.: NZ 1997-328853 A 19970926
WO 1998-NZ145 W 19980925

AB This invention is directed to new therapeutic uses which involve the stimulation of chondrocyte proliferation. More particularly, it is directed to the use of **amylin** and adrenomedullin and their analogs as agents which stimulate **chondrocyte** proliferation and which therefore have utility in the treatment of cartilage disorders

and/or cartilage mediated bone growth. Thus, **amylin**(1-8) (10-8M) stimulated **chondrocyte** proliferation, increasing cell nos. from 3.23 x 104 to 3.63 x 104 as well as increasing thymidine incorporation (i.e. DNA synthesis) from 26859 ± 423 to 28932 ± 628 dpm.

L68 ANSWER 8 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN DUPLICATE 4
ACCESSION NUMBER: 1999:767667 CAPLUS
DOCUMENT NUMBER: 132:90294
TITLE: Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes
AUTHOR(S): Cornish, J.; Callon, K. E.; Lin, C. Q.-X.; Xiao, C. L.; Mulvey, T. B.; Cooper, G. J. S.; Reid, I. R.
CORPORATE SOURCE: Department of Medicine, University of Auckland, Auckland, 1001, N. Z.
SOURCE: American Journal of Physiology (1999), 277(5, Pt. 1), E779-E783
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Peptides purified by HPLC are often in the form of a trifluoroacetate (TFA) salt, because trifluoroacetic acid is used as a solvent in reversed-phase HPLC separation However, the potential effects of this contaminant in culture systems have not been addressed previously. TFA (10-8 to 10-7 M) reduced cell nos. and thymidine incorporation into fetal rat osteoblast cultures after 24 h. Similar effects were found in cultures of articular chondrocytes and neonatal mouse calvariae, indicating that the effect is not specific to one cell type or to one species of origin. When the activities of the TFA and hydrochloride salts of amylin, amylin-(1-8), and calcitonin were compared in osteoblasts, cell proliferation was consistently less with the TFA salts of these peptides, resulting in failure to detect a proliferative effect or wrongly attributing an antiproliferative effect. This finding is likely to be relevant to all studies of purified peptides in concns. above 10-9 M in whatever cell or tissue type. Such peptides should be converted to a hydrochloride or biol. equivalent salt before assessment of their biol. effects is undertaken.
REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L68 ANSWER 9 OF 18 Elsevier BIOBASE COPYRIGHT 2004 Elsevier Science B.V. on STN
ACCESSION NUMBER: 1999278520 ESBIOBASE
TITLE: Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes
AUTHOR: Cornish J.; Callon K.E.; Lin C.Q.-X.; Xiao C.L.; Mulvey T.B.; Cooper G.Z.S.; Reid I.R.
CORPORATE SOURCE: J. Cornish, Dept. of Medicine, Univ. of Auckland, Private Bag 92019, Auckland 1001, New Zealand. E-mail: j.cornish@auckland.ac.nz
SOURCE: American Journal of Physiology - Endocrinology and Metabolism, (1999), 277/5 40-5 (E779-E783), 8 reference(s)
CODEN: AJPMD0 ISSN: 0193-1849
DOCUMENT TYPE: Journal; Article
COUNTRY: United States
LANGUAGE: English
SUMMARY LANGUAGE: English
AB Peptides purified by HPLC are often in the form of a trifluoroacetate (TFA) salt, because trifluoroacetic acid is used as a solvent in reversed- phase HPLC separation. However, the potential effects of this contaminant in culture systems have not been addressed previously. TFA (10.sup.-.sup.8 to 10.sup.-.sup.7 M) reduced cell numbers and thymidine incorporation into fetal rat osteoblast cultures after 24 h. Similar effects were found in cultures of articular **chondrocytes** and

neonatal mouse calvariae, indicating that the effect is not specific to one cell type or to one species of origin. When the activities of the TFA and hydrochloride salts of **amylin**, **amylin**-(18), and calcitonin were compared in osteoblasts, cell proliferation was consistently less with the TFA salts of these peptides, resulting in failure to detect a proliferative effect or wrongly attributing an antiproliferative effect. This finding is likely to be relevant to all studies of purified peptides in concentrations above 10.sup.-.sup.9 M in whatever cell or tissue type. Such peptides should be converted to a hydrochloride or biologically equivalent salt before assessment of their biological effects is undertaken.

L68 ANSWER 10 OF 18 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 1999:883229 SCISEARCH
THE GENUINE ARTICLE: 254EJ
TITLE: Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes
AUTHOR: Cornish J (Reprint); Callon K E; Lin C Q X; Xiao C L; Mulvey T B; Cooper G J S; Reid I R
CORPORATE SOURCE: UNIV AUCKLAND, DEPT MED, PRIVATE BAG 92019, AUCKLAND 1001, NEW ZEALAND (Reprint); UNIV AUCKLAND, SCH BIOL SCI, AUCKLAND 1001, NEW ZEALAND
COUNTRY OF AUTHOR: NEW ZEALAND
SOURCE: AMERICAN JOURNAL OF PHYSIOLOGY-ENDOCRINOLOGY AND METABOLISM, (NOV 1999) Vol. 277, No. 5, pp. E779-E783. Publisher: AMER PHYSIOLOGICAL SOC, 9650 ROCKVILLE PIKE, BETHESDA, MD 20814. ISSN: 0193-1849.
DOCUMENT TYPE: Article; Journal
FILE SEGMENT: LIFE
LANGUAGE: English
REFERENCE COUNT: 8
ABSTRACT IS AVAILABLE IN THE ALL AND IALL FORMATS

AB Peptides purified by HPLC are often in the form of a trifluoroacetate (TFA) salt, because trifluoroacetic acid is used as a solvent in reversed-phase HPLC separation. However, the potential effects of this contaminant in culture systems have not been addressed previously. TFA (10(-8) to 10(-7) M) reduced cell numbers and thymidine incorporation into fetal rat osteoblast cultures after 24 h. Similar effects were found in cultures of articular **chondrocytes** and neonatal mouse calvariae, indicating that the effect is not specific to one cell type or to one species of origin. When the activities of the TFA and hydrochloride salts of **amylin**, **amylin**-(1-8), and calcitonin were compared in osteoblasts, cell proliferation was consistently less with the TFA salts of these peptides, resulting in failure to detect a proliferative effect or wrongly attributing an antiproliferative effect. This finding is likely to be relevant to all studies of purified peptides in concentrations above 10(-9) M in whatever cell or tissue type. Such peptides should be converted to a hydrochloride or biologically equivalent salt before assessment of their biological effects is undertaken.

L68 ANSWER 11 OF 18 EMBASE COPYRIGHT 2004 ELSEVIER INC. ALL RIGHTS RESERVED. on STN DUPLICATE 5
ACCESSION NUMBER: 1999194750 EMBASE
TITLE: Skeletal effects of amylin and related peptides.
AUTHOR: Cornish J.; Reid I.R.
CORPORATE SOURCE: Dr. J. Cornish, Department of Medicine, University of Auckland, Private Bag 92019, Auckland, New Zealand. j.cornish@auckland.ac.nz
SOURCE: Endocrinologist, (1999) 9/3 (183-189). Refs: 18
ISSN: 1051-2144 CODEN: EDOCEB
COUNTRY: United States
DOCUMENT TYPE: Journal; General Review
FILE SEGMENT: 003 Endocrinology
037 Drug Literature Index

LANGUAGE: English
SUMMARY LANGUAGE: English
AB **Amylin** and adrenomedullin are related peptides with some homology to both calcitonin and calcitonin gene-related peptide. Both **amylin** and adrenomedullin recently have been found to stimulate the proliferation of osteoblasts in vitro and to increase indices of bone formation when administered either locally or systemically in vivo. **Amylin** also has been found to act on **chondrocytes**, stimulating their proliferation in culture and increasing tibial growth plate width and tibial length when administered systemically to adult mice. Systemic administration of **amylin** also is associated with increased fat mass, consistent with its known effects on fuel metabolism. However, we recently have established that the osteotropic effects of **amylin** are retained in an octapeptide fragment of the molecule, which has no activity on carbohydrate metabolism. Thus, this small peptide, or analogues of it, are potential candidates as anabolic therapies for osteoporosis. Similar fragments of adrenomedullin, which retain activity on bone but lack the parent peptide's vasodilator properties, also are being defined. In addition to a potential therapeutic role, these peptides may play a part in normal bone physiology. **Amylin** is secreted after eating and may direct calcium and protein absorbed from the meal into new bone synthesis. **Amylin** circulates in high concentrations in obese individuals and might contribute to the association between bone mass and fat mass. Finally, adrenomedullin and its receptor are easily detectable during rodent embryogenesis, suggesting that these peptides also might act as autocrine or paracrine regulators of bone growth. Further research is necessary to confirm these interesting possibilities.

L68 ANSWER 12 OF 18 PASCAL COPYRIGHT 2004 INIST-CNRS. ALL RIGHTS RESERVED. on STN
ACCESSION NUMBER: 2000-0041758 PASCAL
COPYRIGHT NOTICE: Copyright .COPYRGT. 2000 INIST-CNRS. All rights reserved.
TITLE (IN ENGLISH): Trifluoroacetate, a contaminant in purified proteins, inhibits proliferation of osteoblasts and chondrocytes
AUTHOR: CORNISH J.; CALLON K. E.; LIN C. Q.-X.; XIAO C. L.; MULVEY T. B.; COOPER G. J. S.; REID I. R.
CORPORATE SOURCE: Department of Medicine, University of Auckland, Auckland 1001, New Zealand; School of Biological Sciences, University of Aucklan, Auckland 1001, New Zealand
SOURCE: American journal of physiology. Endocrinology and metabolism, (1999), 40(5), E779-E783, 8 refs. ISSN: 0193-1849 CODEN: AJPMD9
DOCUMENT TYPE: Journal
BIBLIOGRAPHIC LEVEL: Analytic
COUNTRY: United States
LANGUAGE: English
AVAILABILITY: INIST-670C1, 354000080366430010
AN 2000-0041758 PASCAL
CP Copyright .COPYRGT. 2000 INIST-CNRS. All rights reserved.
AB Peptides purified by HPLC are often in the form of a trifluoroacetate (TFA) salt, because trifluoroacetic acid is used as a solvent in reversed-phase HPLC separation. However, the potential effects of this contaminant in culture systems have not been addressed previously. TFA (10.sup.-.sup.8 to 10.sup.-.sup.7 M) reduced cell numbers and thymidine incorporation into fetal rat osteoblast cultures after 24 h. Similar effects were found in cultures of articular **chondrocytes** and neonatal mouse calvariae, indicating that the effect is not specific to one cell type or to one species of origin. When the activities of the TFA and hydrochloride salts of **amylin**, **amylin**-(1-8), and calcitonin were compared in osteoblasts, cell proliferation was consistently less with the TFA salts of these peptides, resulting in failure to detect a proliferative effect or wrongly attributing an antiproliferative effect. This finding is likely to be relevant to all

studies of purified peptides in concentrations above 10.sup.-.sup.9 M in whatever cell or tissue type. Such peptides should be converted to a hydrochloride or biologically equivalent salt before assessment of their biological effects is undertaken.

L68 ANSWER 13 OF 18 PHIN COPYRIGHT 2004 PJB on STN

ACCESSION NUMBER: 1998:15417 PHIN
DOCUMENT NUMBER: B00592190
DATA ENTRY DATE: 1 Jul 1998
TITLE: The Phase III Club
SOURCE: Bioventure-View (1998) No. 1307 p4
DOCUMENT TYPE: Newsletter
FILE SEGMENT: FULL

L68 ANSWER 14 OF 18 CAPLUS COPYRIGHT 2004 ACS on STN
ACCESSION NUMBER: 1998:679141 CAPLUS
DOCUMENT NUMBER: 130:20821
TITLE: Systemic administration of amylin increases bone mass, linear growth, and adiposity in adult male mice
AUTHOR(S): Cornish, Jillian; Callon, Karen E.; King, Alan R.; Cooper, Garth J. S.; Reid, Ian R.
CORPORATE SOURCE: Department of Medicine, University of Auckland, Auckland, 92019, N. Z.
SOURCE: American Journal of Physiology (1998), 275(4, Pt. 1), E694-E699
CODEN: AJPHAP; ISSN: 0002-9513
PUBLISHER: American Physiological Society
DOCUMENT TYPE: Journal
LANGUAGE: English
AB Amylin is a peptide hormone cosecreted with insulin from the pancreatic β-cells that can act as an osteoblast mitogen and as an inhibitor of bone resorption. The effects on bone of its systemic administration are uncertain. The present study addresses this question in adult male mice that were given daily s.c. injections of amylin (10.5 μg) or vehicle for 4 wk. Histomorphometric indexes of bone formation increased 30-100% in the amylin-treated group, whereas resorption indexes were reduced by .apprx.70%. Total bone volume in the proximal tibia was 13.5% in control animals and 23.0% in those receiving amylin. Cortical width, tibial growth plate width, tibial length, body weight, and fat mass were all increased in the amylin-treated group. It is concluded that systemic administration of amylin increases skeletal mass and linear bone growth. This peptide has potential as a therapy for osteoporosis if its bone effects can be dissociated from those on soft tissue mass.
REFERENCE COUNT: 28 THERE ARE 28 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L68 ANSWER 15 OF 18 DISSABS COPYRIGHT (C) 2004 ProQuest Information and Learning Company; All Rights Reserved on STN
ACCESSION NUMBER: 95:2733 DISSABS Order Number: AAR9430910
TITLE: ROLES OF THE NUCLEATIONAL CORE COMPLEX AND COLLAGENS (TYPE II AND X) IN CALCIFICATION OF GROWTH PLATE MATRIX VESICLES AND STUDIES ON CALCIFYING CHONDROCYTES IN CULTURE
AUTHOR: MWALE, FACKSON [PH.D.]; ISHIKAWA, YOSHINORI [advisor]
CORPORATE SOURCE: UNIVERSITY OF SOUTH CAROLINA (0202)
SOURCE: Dissertation Abstracts International, (1994) Vol. 55, No. 7B, p. 2710. Order No.: AAR9430910. 230 pages.
DOCUMENT TYPE: Dissertation
FILE SEGMENT: DAI
LANGUAGE: English
ENTRY DATE: Entered STN: 19950111
Last Updated on STN: 19950111
AB Matrix vesicles (MV) have been shown to initiate mineralization in cartilage and other vertebrate tissues. However, little is known about the factors that regulate mineralization of MV. Recent studies have shown that a preformed nucleational core which mainly consists of Ca$\sp{2+}$-PS-Pi

complex, is necessary for the rapid accumulation of Ca$\sp{2+}$ by MV in vitro. In this comparative study, three different enzyme digestion methods are used to release MV: TCRMV (trypsin/collagenase), HRMV (hyaluronidase), or HCRMV (hyaluronidase/collagenase), TCRMV pellets contained type II and X collagens, while HRMV and HCRMV did not, and only TCRMV showed a high uptake of Ca$\sp{2+}.$ However, binding of native type II collagen stimulated HRMV and HCRMV uptake of Ca$\sp{2+}.$

Our recent development of cultures of epiphyseal growth plate chondrocytes that are capable of mineralizing in the absence of b-glycerophosphate provides a useful model for studying the direct effect of osteotropic agents on skeletal cells. The chondrocytes reach confluence and become hypertrophic after 2 weeks in culture, after which they form nodules and cellular blebs and then induce mineral deposition. After treatment with sodium hypochlorite, the mineralized cell layer revealed numerous calcospherite-like structures arranged in the concave lacunar wall. This is the first time these structures have been observed in culture.

The regulatory function of **amylin** (new member of calcitonin/CGRP) on mineralization of growth plate **chondrocytes** and collagen synthesis was studied. **Amylin** stimulates alkaline phosphatase activity and mineral formation at early time points. **Amylin** binds to annexin V which, in turn, acts as a calcium channel in MV. Rat **amylin** fragment (8-37), however, showed no effect on mineralization of **chondrocytes** and did not bind to annexin V, indicating a possible role for the NH$\sb2$-terminal region of **amylin** for biological activity. **Amylin** also stimulates type II collagen synthesis in sternal **chondrocytes** in serum-free medium. These findings implicate **amylin** in processes regulating endochondral bone formation.

L68 ANSWER 16 OF 18 BIOSIS COPYRIGHT 2004 BIOLOGICAL ABSTRACTS INC. on STN
ACCESSION NUMBER: 1993:336401 BIOSIS
DOCUMENT NUMBER: PREV199345031126
TITLE: A possible regulatory function of **amylin** on the mineralization of growth plate **chondrocytes**.
AUTHOR(S): Mwale, Fackson; Kirsch, Thorsten; Ishikawa, Yoshinori; Wuthier, Roy
CORPORATE SOURCE: Dep. Chem. Biochem., Univ. S.C., Columbia, SC 29208, USA
SOURCE: FASEB Journal, (1993) Vol. 7, No. 7, pp. A1238. Meeting Info.: Joint Meeting of the American Society for Biochemistry and Molecular Biology and American Chemical Society Division of Biological Chemistry. San Diego, California, USA. May 30-June 3, 1993. CODEN: FAJOEC. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; (Meeting)
LANGUAGE: English
ENTRY DATE: Entered STN: 16 Jul 1993
Last Updated on STN: 17 Jul 1993

L68 ANSWER 17 OF 18 SCISEARCH COPYRIGHT 2004 THOMSON ISI on STN
ACCESSION NUMBER: 93:272095 SCISEARCH
THE GENUINE ARTICLE: KY848
TITLE: A POSSIBLE REGULATORY FUNCTION OF **AMYLIN** ON THE MINERALIZATION OF GROWTH PLATE **CHONDROCYTES**
AUTHOR: MWALE F (Reprint); KIRSCH T; ISHIKAWA Y; WUTHIER R
CORPORATE SOURCE: UNIV S CAROLINA, DEPT CHEM & BIOCHEM, COLUMBIA, SC, 29208
COUNTRY OF AUTHOR: USA
SOURCE: FASEB JOURNAL, (20 APR 1993) Vol. 7, No. 7, pp. A1238. ISSN: 0892-6638.
DOCUMENT TYPE: Conference; Journal
FILE SEGMENT: LIFE
LANGUAGE: ENGLISH
REFERENCE COUNT: No References

L68 ANSWER 18 OF 18 DGENE COPYRIGHT 2004 THOMSON DERWENT on STN
ACCESSION NUMBER: AAY01705 peptide DGENE

TITLE: Treating patient to stimulate **chondrocyte** proliferation in vivo comprising administration of **amylin**, adrenomedullin or ligand growth to stimulate receptor useful for cartilage/bone repair
INVENTOR: Cornish J; Reid I R
PATENT ASSIGNEE: (AUCK-N)AUCKLAND UNISERVICES LTD.
PATENT INFO: WO 9916406 A2 19990408 25p
APPLICATION INFO: WO 1998-NZ145 19980925
PRIORITY INFO: NZ 1997-328853 19970926
DOCUMENT TYPE: Patent
LANGUAGE: English
OTHER SOURCE: 1999-277029 [23]
DESCRIPTION: Peptide sequence of amylin.
AN AAY01705 peptide DGENE
AB The present sequence represents an **amylin** protein. The specification describes a method for increasing the active concentration of **amylin**, adrenomedullin or ligand receptor within a patient to stimulate **chondrocyte** proliferation. The method is useful for treating a patient to stimulate **cartilage growth** and repair and bone growth (especially effecting the lineal growth of bone) in vivo through stimulation of **chondrocyte** proliferation.

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